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A Glucose Electrochemical Sensor Based on Glucose Oxidase Immobilized in Aminopropyl-Magnesium Phyllosilicate Nanosheets Matrix

Li-Min Liu[*], *Fang-Yuan Liu, Xue-Xia Liu, Chun-Fang Huang, Zhi-Jun Wang, De-Yong He*

School of Chemistry and Chemical Engineering, the Key Laboratory of Coordination Chemistry of Jiangxi Province, Institute of Applied Chemistry, Jinggangshan University, Ji'an, Jiangxi 343009, China
[*]E-mail: llm24@126.com

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In this work, glucose oxidase (GOD) was immobilized in aminopropyl-magnesium phyllosilicate (AMP) nanosheets matrix to obtain the GOD/AMP composite *via* an exfoliation-assembly procedure, and a glucose biosensor was constructed by dropping the GOD/AMP composite and nafion film successively onto the glassy carbon electrode surface. The direct electrochemical behavior, the electrocatalytic response to dissolved oxygen and glucose, as well as the ability to detect glucose in real sample were investigated by the methods of cyclic voltammetry and chronoamperometry. The results indicate that the immobilization of GOD in AMP nanosheets matrix maintained the biological activity and facilitated the direct electron transfer of GOD on the glassy carbon electrode surface. The GOD/AMP-composite modified electrode show a sensitively response to glucose, with the linear range of 1.0×10^{-4} to 1.1×10^{-3} molL^{-1}and the detection limit of 0.035 mmol L^{-1}. Moreover, the glucose can be detected directly in the real sample of blood serum by this GOD/AMP composite modified electrode.

Keywords: Aminopropyl-magnesium phyllosilicate (AMP), Glucose Oxidase (GOD), Electrochemical biosensor, Direct electrochemistry, Glucose

1. INTRODUCTION

The direct electrochemistry and the catalytic-activity of biomolecules on the based electrode were the key factors to the performance of the third-generation biosensors. As indicated in the previous reports, immobilization biomolecules into the matrix of inorganic nanomaterials can often enhance the direct electron transfer between the redox enzyme and the based electrode, such asnanoparticles (gold [1], silica [2], TiO_2 [3], Fe_3O_4 [4], MnO_2 [5], $CaCO_3$[6] and clay [7-9] nanoparticles et al.), nanotubes (carbon [10] and TiO_2 [11]nanotubes et al.), nanorods[12], nanofiber[13], nanoflowers[14]and nanocomposites [15-18].

Recently, layered inorganic materials show especially attractivein electrochemistry due to their notable advantagesthan other inorganic nanomaterials with spherical ortubular structure. Firstly, the biomacromolecules can intercalate into the expandable interlayers of layered materials *via* ion-exchange method or delamination-assembly procedure, overcoming the limitation of large size biomolecules absorbed only on the surface of other inorganic nanomaterials[19-23]. Secondly, immobilization of biomolecules into the interlayers could enhance often their activity and improved their thermal/chemical stability[24-26]. Thirdly, immobilization of biomolecules in the nanosheets matrix of exfoliated layered solids facilitated to the direct electrochemistry of redox enzyme on based electrode usually[27-30].

However, the efficiency and effect of biomolecules combined with layered inorganic materials was due to the charge match and the interaction strength between host layer and guest molecules. In order to control the nature as well as the strength of host-guest interactions, those layered materials with inorganic-organic hybrid structure were investigated recently, by decoratingthe layered host with appropriate functional groups[31-33]. For example, Mann et al. reported the intercalating of DNA directly into aminopropyl-functionalized magnesium phyllosilicate (AMP) with positive charge layers [34].Kumar et al. synthesized a variety of zirconium phosphonates by introducing carboxymethyl- and carboxyethyl- groups to the host layer of α-zirconium phosphates, and investigated systematically the protein-solid interaction[35]. We also obtained anaminoethoxy-functionalized zirconium phosphonate, abbreviated as ZrRP, by decorating the aminoethoxy- group on the α-zirconium phosphateand achieved the DNA intercalated directly into layered zirconium phosphates/zirconium phosphonates firstly[36].

In this work, glucose oxidase (GOD) was immobilized in Aminopropyl-magnesium phyllosilicate (AMP) nanosheets matrix to obtain the GOD/AMP composite *via* an exfoliation-assembly procedure, and a glucose biosensor was constructed by dropping the GOD/AMP composite and nafion film successively onto the glassy carbon electrode surface. The direct electrochemical behavior, the electrocatalytic response to dissolved oxygen and glucose, as well as the ability to detect glucose in real sample were investigated. The results indicate that the immobilization of GOD in AMP nanosheets matrix maintained the biological activity and facilitated the direct electron transfer of GOD on the glassy carbon electrode surface. The GOD/AMP composite modified electrode show a sensitively electrocatalytic response to dissolved oxygen and glucose, and can be applied to the glucose detection in the real sample of human serum.

2. EXPERIMENTAL METHOD

2.1. Materials

Glucose oxidase (GOD) and perfluorinated ion resin (nafion)were purchased from the Sigma Chemical Co. 3-aminopropyltriethoxysilane was obtained from Aladdin Chemical Co. All other reagents were of analytical grade, and the water used in the experiments was deionized.

2.2. Synthesis and delamination of aminopropyl-magnesium phyllosilicate

The aminopropyl-magnesium phyllosilicate, abbreviated as AMP, was prepared according to the reference [34]as follow,0.84 g of magnesium chloride hexahydrate was dissolved in 20 gethanol, then 1.3 mL of 3-aminopropyltriethoxysilanewas added dropwise to this solution with continuous stirring. The white slurry obtained after 5 minutes was stirred overnight and the precipitate isolated by centrifugation, then washed with ethanol(50 mL) and dried at 40 °C.

To delaminate AMP, 20 mg of AMP was sonicated in 2.0 mL aqueous suspension for 10 minutes. The final concentration of the exfoliated AMP was approximately 10 mg mL^{-1}.

2.3. Preparation of GOD /AMP composites

The GOD/AMP composites was obtained by dispersing 10 mg of the GOD into the 1.0 mL AMP suspension prepared above, and then the final concentration of GOD in the AMP suspension was approximately 10 mg mL^{-1}.

2.4. Construction of the biosensors

Glassy carbon electrodes polished with 1, 0.3, and 0.05 μm alumina powder, followed by sonicating in acetone and doubly distilled water sequentially. Then, the electrodes were dried under a stream of nitrogen gas. After shaking for 5 minutes, 5μL of the GOD/AMP composites suspension was dropped onto the surface of a glassy carbon electrode and dried at room temperature, then, 5μL of the nafion was dropped onto the surface of GOD/AMP composite.

2.5. Measurements

The UV-Vis (UV) absorption spectra were detected by a Centra 10 spectrophotometer(GBC, Australia). The electrochemical measurements were conducted on a CHI660E workstation (Shanghai Chenhua, China), with a saturated calomel electrode as the reference, a platinum wire as the counter electrode and the modified glassy carbon electrode (GCE) as the working electrode. The dissolved oxygen in buffer solutions were purged by nitrogen stream before the electrochemical experiments, and the anaerobic environment was maintained in the cell by continuously bubbling nitrogen during the course of the experiments.

2.6 Detection of the glucose in human serum

In this work, the glucose level was determined as follow. The cyclic voltammograms (CVs) was recorded in the PBS buffer solution with air-saturated, then the standard sample of glucose were added successively into the air-saturated buffer solution with the concentration of 0.1, 0.2, 0.3, 0.4mmol L^{-1} and recorded the CVs respectively, then 50, 100, 150μLhuman serum were added into the buffer solution with glucose concentration of 0.4mmol L^{-1} and recorded the CVs respectively. The

glucose level in human serum was calculated according to the decrease of the cathodic peak current at -450mV of each CV.

In hospital, the glucose in human serum was catalyzed by glucose oxidase to produce gluconic acid and hydrogen peroxide, then hydrogen peroxide reacted with 4-aminoantipyrineand phenol under the catalysis of peroxidase to producequinone imine. The glucose level in human serum was then calculated according to the absorbance of quinone imine at 500nm.

3. RESULTS AND DISCUSSION

3.1. UV–vis characteristication of the GOD/AMP composite

The results of UV–vis spectra of the native GOD, exfoliated AMP nanosheets and GOD/AMP composite were shown in figure 1. In the wavelength range from 220 nm to 400 nm, native GOD exhibited the characteristic absorption at 274 nm in curve a, however, no obvious absorbance band could be observed for the exfoliated AMP nanosheets in curve b. After GOD was combined with AMP nanosheets to form the GOD/AMP composite, the characteristic band of immobilized GOD is almost unchanged compared to that of the native GOD, only the absorbance intensity was weaken slightly, as shown in curve c. The results indicated GOD maintained the secondary structure almost after combined with the AMP nanosheets.

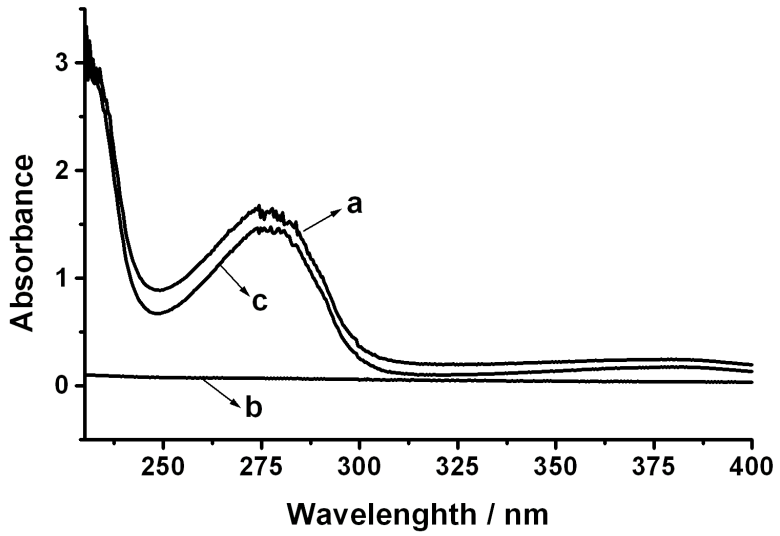

Figure 1. The UV spectra of the native GOD (a), exfoliated AMP nanosheets (b) and GOD/AMP composite (c).

3.2. Direct electrochemistry of the GOD/AMP composite modified electrode

The cyclic voltammograms (CVs) of native GOD and GOD/AMPcomposite modified electrode were shown in figure 2. A couple of reversible and well-defined redox peaks at -409 and -449 mV were observed on the GOD/AMP/nafion electrode, whereas the weakened redox peaks at -345 and -

395 mV were exhibited on the GOD/nafion electrode. The shift of the apparent formal peak potential (E_p) from -370 mV on the GOD/nafion electrode to -429 mV on the GOD/AMP/nafion electrode, maybe due to the difference of native and immobilized GOD. The decrease of the peak-to-peak separation ($\triangle E_p$) from 50 mV on the GOD/nafion electrode to 40 mV on the GOD/AMP/nafion electrode, suggested that the GOD direct electrochemistry was enhanced by immobilizing native GOD into the matrixs of AMP nanosheets. The apparent formal peak potential (E_p) of this GOD/AMP-GCE was close to the value of the GOD/GR-GO/GCE [37] and the GOD/m-TDNF/Nafion/GCE [38], attributed to the direct electrochemistry of GOD for the conversion of GOD(FAD) to GOD($FADH_2$).

The effect of the scan rate on the response of the GOD/AMP composite electrode was investigated. As shown in figure 3, both the reduction and oxidation peak currents (i_p) increased linearly with the increase of scan rate from 0.05 to 0.3V s^{-1}, suggesting a surface-controlled process. The electron transfer rate constant, k_s, can be estimated by the formula $k_s = mnFv/RT$ if the peak-to-peak separation is less than 200mV, where m is a parameter related to the peak-to-peak separation, n is the number of transferred electron, F is the Faraday's constant, v is the scan rate, T is the temperature and R is the universal gas constant. The average k_s value was calculated to be 5.26 $s^{-1}$of the GOD/AMP composite electrode and 3.26 s^{-1} of the native GOD modified electrode respectively, according to Laviron's theory [39]. The electron transfer rate constant (k_s) of this GOD/AMP-GCE was comparable to the value of the GOD/chitosan/α-ZrP/GCE [30] and the RGO/Ag/GOx-GCE [40], and faster than the value of the GOD/GR-GO/GCE [37], suggested the electron communication between GOD and glassy carbon electrode was enhanced remarkably by the AMP nanosheets.

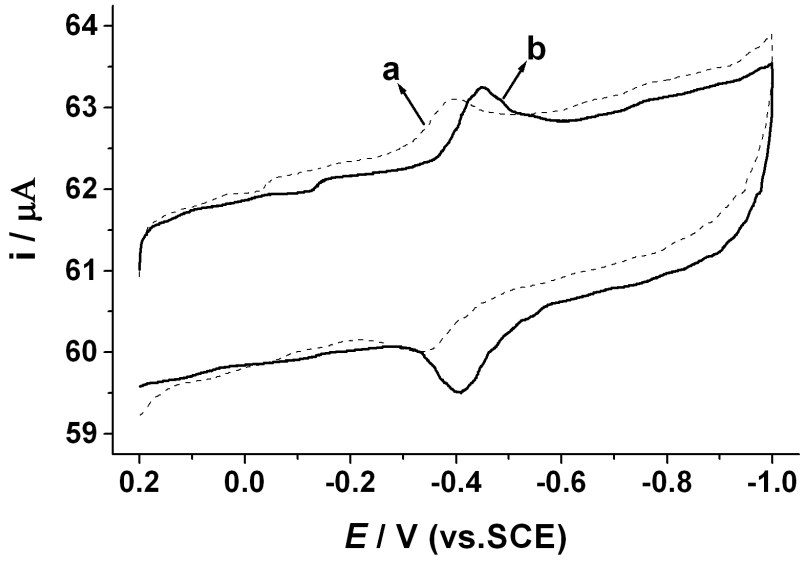

Figure 2. The CVs of the native GOD (a) and the GOD/AMP composite (b) modified electrode in 0.1mol L^{-1} PBS buffer solution (pH 7.02) at scan rate 100 mV s^{-1}.

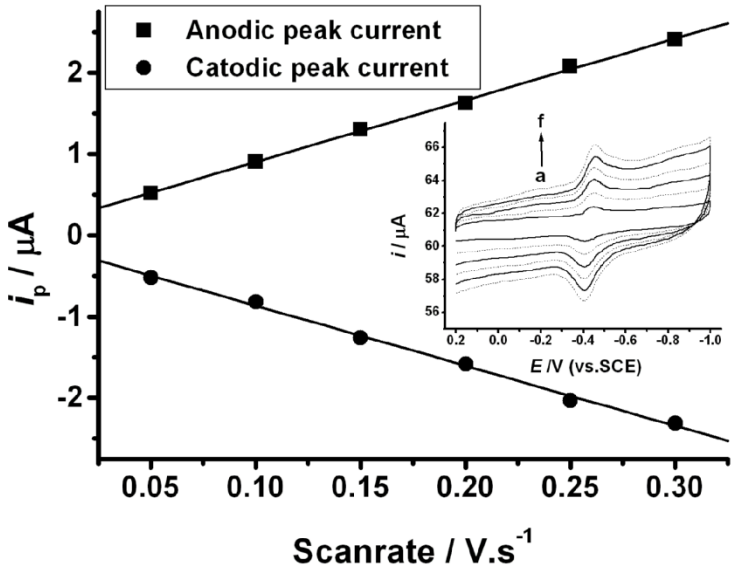

Figure 3. The influence of the scan rate (0.1 to 1.0V s^{-1}) on the cathodic and anodic peak currents of the GOD/AMP composite modified electrode in 0.1mol L^{-1} PBS buffer solution at pH 7.02, and the CVs of the GOD/AMP composite modified electrode at different scan rate from 0.1 to 1.0V s^{-1} (Inset).

3.3. Electrocatalytic properties of the GOD/AMP composite modified electrode and glucose detection

The CVs of the GOD/AMP composite modified electrode in the anaerobic and air-saturated PBS buffer solution were shown in figure 4. A couple of reversible and well-defined redox peaks was observed in the anaerobic buffer solutions, due to the reversible reaction between GOD-FAD and GOD-$FADH_2$ (Eq. 1). However, the cathodic peak current is increased and the anodic peak current is decreased obviously in the air-saturated solution, attributing to the oxygen reduction catalyzed by the immobilized GOD in the AMP (Eq. 2).

When glucose was added to this air-saturated PBS buffer solution, the cathodic peak current decreased due to the oxidation of glucose catalyzed by GOD and the consuming of dissolved oxygen (Eq. 3), as shown in figure5. Based on the decrease of the dissolved oxygen and the cathodic peak current, this GOD/AMP composite modified electrode was applied as a glucose biosensor. The linear relationship between the decrease of cathodic peak currentat -450mV with glucose concentration was $i_{decreased}(\mu A) = 1.96C_{glucose} + 0.08$in the range of 0.1~1.1mmol L^{-1}(R = 0.9993, n = 11), and at -700mV was $i_{decreased}(\mu A) = 5.51C_{glucose} - 0.026$ in the range of 0.1~1.1mmol L^{-1}(R = 0.9999, n = 11), with a detection limit of 0.086 mmol L^{-1} and 0.035 mmol L^{-1}respectively.

$$GOD - FAD + 2e^- + 2H^+ \rightarrow GOD - FADH_2 \quad (1)$$

$$GOD - FADH_2 + O_2 \rightarrow GOD - FAD + H_2O_2 \quad (2)$$

$$glucose + O_2 \xrightarrow{GOD} gluconolactone + H_2O_2 \quad (3)$$

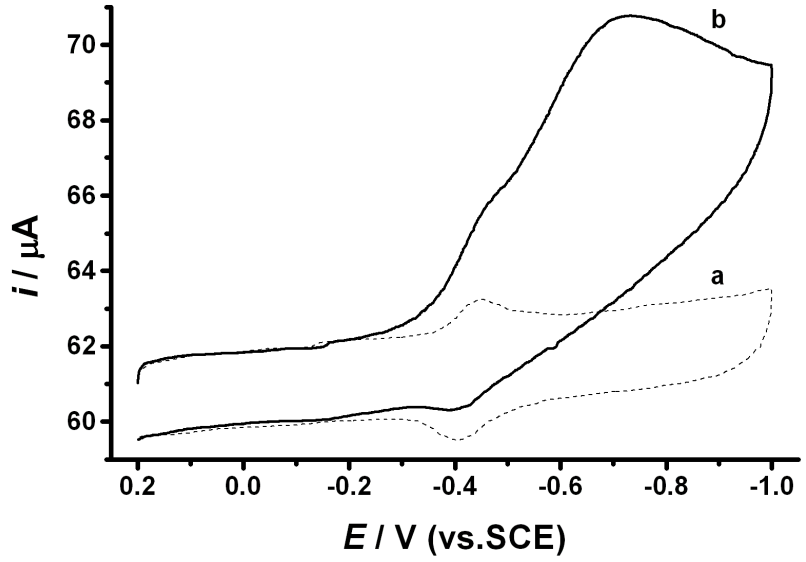

Figure 4. The CVs of the GOD/AMP composite modified electrode in anaerobic (a) and air-saturated PBS buffer solution (0.1mol L^{-1}, pH 7.02).

The amperometric response of the GOD/AMP composite modified electrode to glucose was shown in figure 6. Each successive addition of glucose into the air-saturated and stirred PBS buffer solution caused a decrease in the steady-state current. The linear relationship between the decrease of the steady-state current at –700 mV with glucose concentration was $i_{decreased}(\mu A) = 3.20C_{glucose} + 0.021$($R$ = 0.9966, n = 12), in range of 0.1~1.2mmol L^{-1}. This phenomena is according well to the result obtained by the CV characterization, indicating the higher retain of enzymatic activity of GOD in the matrix of AMP nanosheets.

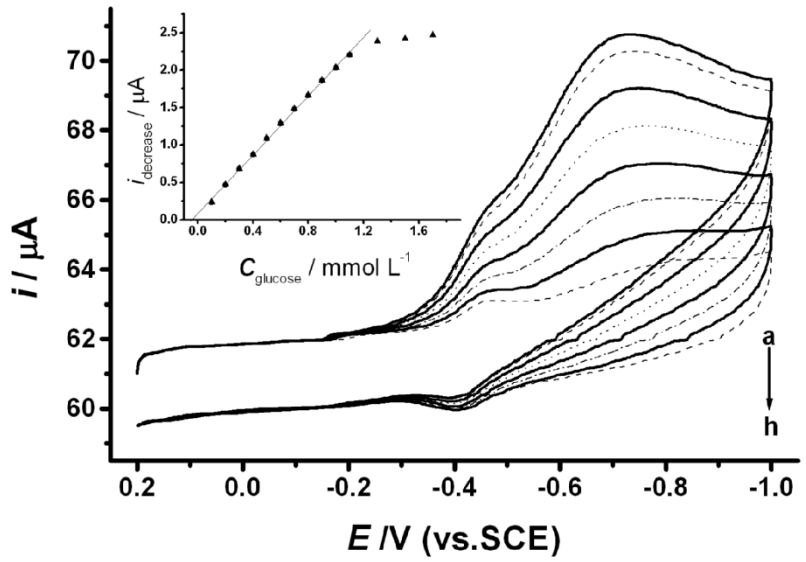

Figure 5. The CVs of the GOD/AMP composite modified electrode in air-saturated (a) PBS buffer solution, air-saturated with 0.1 (b), 0.3 (c), 0.5 (d), 0.7 (e), 0.9 (g), 1.1(f) and 1.7 (h) mmol L^{-1}glucose(in 0.1 mol L^{-1} PBS, pH 7.02), the plots of the decrease of cathodic peak current at -450mV versus glucose concentration for the modified electrode (Insert).

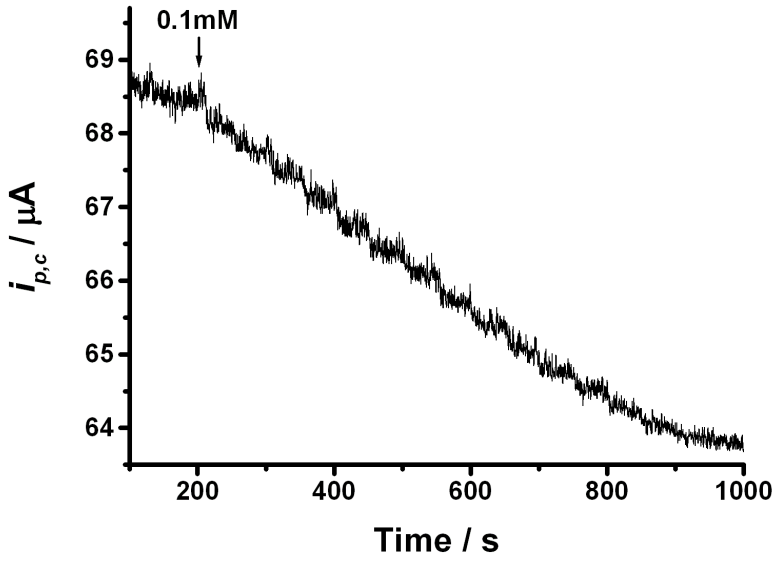

Figure 6. The amperometric response of the GOD/AMP composite modified electrode at -700mV in PBS buffer solution (0.1mol L^{-1}, pH 7.02) after the successive addition 0.1 mmol L^{-1} of glucose.

The comparison of analytical parameters of the GOD/AMP composite modified electrode with some other glucose biosensor was shown in table 1. The linear range and the detection limit of this GOD/AMP composite modified electrode is better than the GOD immobilized in the "α-ZrP nanosheets/chitosan" [30], "graphene-graphene oxide" [37], "reduced graphene oxide/silver nanoparticles" [40], "glutaraldehyde/molybdenite" [41], "gold nanoparticles/carbon nanotubes/PVA" [42] modified glassy carbon electrode, but not as good as the GOD immobilized in the "gold nanoparticles/polyaniline/multi-walled carbon nanotubes" [43], "multi-walled carbon nanotubes/cobalt(II) sulfide nanoparticles" [44], "zinc oxidenanoparticles /multi-walled carbon nanotubes" [45] and "prussian blue/carbon nanotubes/ionic liquid" [46] modified glassy carbon electrode.

Table 1. Comparison of analytical parameters of the GOD/AMP modified electrode with some other glucose biosensor.

Structure of biosensor	Linear range (mM)	Detection limit (mM)	Reference
GOD/chitosan/α-ZrP/GCE	0.25~8.0	0.076	30
GOD/GR-GO/GCE	0.1-11	0.02	37
GOD/m-TDNF/Nafion/GCE	0.005-3.3	0.0009	38
RGO/Ag/GOx-GCE	0.5-12.5	0.16	40
GOD/GA/MLN/GC	1.0-135	0.1	41
AuNPs–GOD–MWCNTs–PVA/GCE	0.5–8	0.2	42
PTFE/GOx/AuNPs/PANI/MWCNTs/GCE	0.0625-1.19	0.019	43
GOx/CoS-MWCNTs/GCE	0.008-1.5	0.005	44
GOx/ZnO/MWCNTs/GCE	0.00667–1.29	0.00222	45
GOx-PB-BG/GCE	0.0005-0.83	0.0005	46
Nafion-GOD/AMP-GCE	0.1~1.1	0.086/0.035	This work

For the electrochemical determination of glucose, the interferences of some electroactive species which coexist with glucose in human serum must be considered. In an air-saturated and stirred 0.1MpH7.02 PBS buffer solution, when glucose concentration was higher than 0.1 mM, the response arising from 0.05 mmol L^{-1} ascorbic acid (AA), 0.15 mmol L^{-1} urea and 0.025 mmol L^{-1} uric acid (UA) is negligible.

The application of this GOD/AMP modified electrode for biological sample estimation of glucose was explored. For a human serum sample of diabetic, the glucose level was determined by cyclic voltammetry according to the decrease of cathodic peak current at -450mV, and the results were shown in table 2 and figure 7.

Table 2. Glucose levels of a human serum sample of diabetic estimated by the GOD/AMP/nafion multi-layers modified electrode

No	Detected value by this method (mM)	Detected value by hospital (mM)	Relative error (%)
1	9.17	9.76	-6.05
2	9.34		-4.30
3	8.84		-9.43
Average	9.12		-6.60

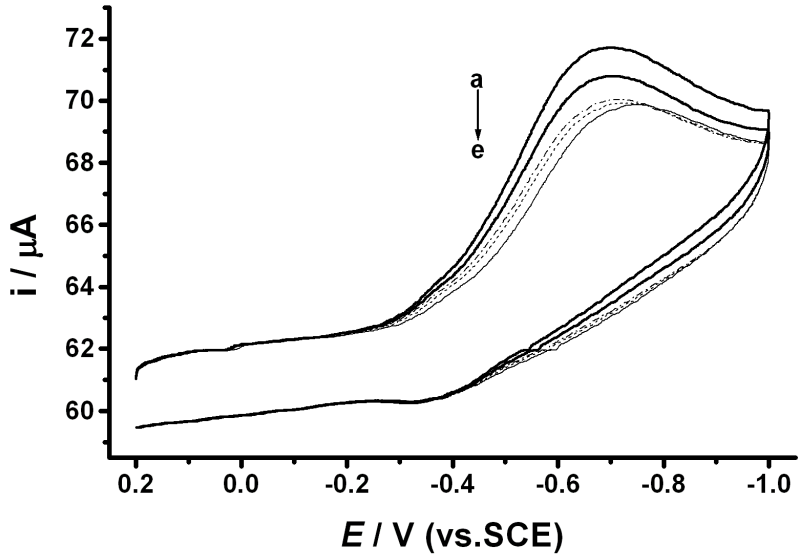

Figure 7. The CVs of the GOD/AMP composite modified electrode in PBS buffer solution (0.1 mol L^{-1}, pH 7.02) with air-saturated (a), air-saturated with0.2 mmol L^{-1}(b) and 0.4 mmol L^{-1} (c) glucose,air-saturated with 0.4mmol L^{-1} glucose and 50μL (g), 150μL (e) human serum.

4. CONCLUSIONS

In summary, glucose oxidase was immobilized in Aminopropyl-magnesium phyllosilicate nanosheets matrix to obtain the GOD/AMP composite *via* an exfoliation-assembly procedure, and a

glucose biosensor was constructed by dropping the GOD/AMP composite and nafion film successively onto the glassy carbon electrode surface. The good performances, including the fast and direct electron transfer of GOD, the sensitivity electrocatalytic response to dissolved oxygen, and the direct detection of glucose in real biological sample, indicated this GOD/AMP composite may be a promising candidate for constructing the third generation glucose biosensor.

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